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Adverse effects of artificial illumination on bat drinking activity

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Running title: Effects of artificial illumination on drinking bats

Abstract

Artificial illumination at night (ALAN) alters many aspects of animal behaviour. Commuting and foraging bats have been found to be affected by ALAN, but no study has yet addressed the impact of lighting on drinking activity, despite its critical importance for bats. We experimentally illuminated cattle troughs used by drinking bats at four forest sites in Italy, and compared drinking activity and foraging activity under lit and dark conditions. We predicted that 1) the number of bat

species and drinking events will be lower under illumination and 2) forest bat species will be more affected than edge specialists. We recorded 2549 drinking events from 12 species or species groups, most of which decreased drinking activity under illumination. The effects of ALAN on drinking were stronger than on foraging. Forest species never drank when the light was on. Edge-foraging species reduced drinking activity while also increasing foraging under lit conditions. We highlight a previously overlooked negative effect of ALAN on bats, whose implications may be locally catastrophic. Given the importance of water sites for both bat foraging and drinking, their illumination should be forbidden, appropriately mitigated or, if necessary, compensated for with the creation of alternative drinking sites.

Key-words: Aquatic habitat; Chiroptera; dehydration; foraging; LED; light pollution

Introduction

Natural light regulates fundamental aspects of animal physiology, ecology and behaviour. Circadian rhythms and seasonal phenology of many species are finely tuned according to the timing, intensity and spectral composition of natural light, which exert major influences on animal survival and fitness (e.g. Rich and Longcore, 2006). Over the last 100 years, artificial illumination at night (ALAN) has progressively expanded in association with ever-increasing urbanization of terrestrial environments globally (Gaston *et al.*, 2015). So far, ALAN has affected large areas of the earth and the process keeps growing at an impressive pace (Hölker *et al.*, 2010). It is estimated that ALAN affects 23% of land surfaces comprised between 75°N and 60°S, almost 90% of Europe, and half of the US (Falchi *et al.*, 2016). Studies conducted so far on the effects of ALAN on biota (e.g. Rich and Longcore, 2006) highlight that it is one of the most striking human-driven environmental alterations, resulting in profound structural and functional interferences with biodiversity (Gaston *et al.*, 2015). By disrupting the natural spatial and temporal distribution of light, artificial illumination affects key aspects of animal physiology with significant – often detrimental – consequences for conservation (Gaston and Bennie, 2014).

Among its many behavioural effects, ALAN may interfere with predation, but this aspect has so far received little attention. An extreme case is given by the suppression of antipredatory responses in tympanate moths caused by ALAN. Moths become easy prey for bats near street lamps as they are less prone to perform the evasive manoeuvres triggered by the detection of echolocation calls that in the dark allow them to avoid predation (Svensson and Rydell, 1998; Tomassini *et al.*, 2014; Wakefield *et al.*, 2015). More generally, artificial light is likely to favour predators that rely on visual cues to locate and capture prey; the latter, on the other hand, will be exposed to a higher mortality risk and greater energy expenditure in vigilance behaviour (Becker *et al.*, 2013; Yorzinski *et al.*, 2015). Although extremely interesting and important for their conservation implications, such aspects have so far received little attention.

As nocturnal mammals, bats are sensitive to light: their nocturnality has probably evolved to reduce the risk of falling victim to predators that hunt by vision such as diurnal birds of prey (e.g. Speakman, 1991a, b; Speakman *et al.*, 1994; Mikula *et al.*, 2016). For instance, roost emergence timing is best explained as a compromise between the need to start foraging at high light levels, i.e. when prey is more abundant, and the increased risk of predation posed under such circumstances by diurnal birds of prey (Speakman, 1991a, b; Jones and Rydell, 1994). Cluttered vegetation surrounding roosts may allow earlier emergence by sheltering bats from aerial attacks of predators (Russo *et al.*, 2007; 2011a). Predation risk is most probably also the reason diurnal flights in bats are so rare (Speakman, 1995). The exception that proves the rule is represented by oceanic islands, often depauperate in birds of prey, where some bat species regularly exhibit daytime activity as a result of release from predators (Speakman, 1995; Russo *et al.*, 2011a; b), though it is unclear why on the same islands other species remain strictly nocturnal. In cluttered habitats, where bats are sheltered from aerial predators, foraging activity may be performed well before sunset (Russo *et al.*, 2011a). Finally, perceived predation risk probably explains lunar phobia in several bat species whose foraging activity correlates negatively with moonlight intensity (Saldaña-Vázquez and Munguía-Rosas, 2013), although reduced prey availability has been proposed as an alternative causal factor.

Hence it is not surprising that most bats show adverse reactions to ALAN, including reduced foraging or commuting activity, avoidance of illuminated sites and roost abandonment (reviewed in Stone *et al.*, 2015a; Mathews *et al.*, 2015; Rowse *et al.*, 2016). Roadside lighting may also reinforce the barrier effect roads have on bats (Fensome and Mathews, 2016). Some bat species tolerate ALAN and often forage on insects attracted to street lights (Rydell, 2006; Polak *et al.*, 2011; Tomassini *et al.*, 2014; Russo and Ancillotto, 2015; Schoeman, 2016; but see Mathews *et al.*, 2015). These species are also often tolerant of urbanisation. Light intolerant species typically have low aspect ratios and wing loadings, traits linked to increased extinction risk (Jones *et al.*, 2003;

Safi and Kerth, 2004) and the ecosystem services they provide may be compromised (Lewanzik and Voigt, 2014), by ALAN.

So far no study has assessed the effect of ALAN on drinking by bats. Bats lose much water through their body surface, particularly via the respiratory system and the wing membranes (Russo *et al.*, 2012; Muñoz-Garcia *et al.*, 2016), so they need to drink regularly to rehydrate (Korine *et al.*, 2016). Bats drink on the wing and do so by performing skilful manoeuvres to contact the water surface with their mouth without crashing into it (Russo *et al.*, 2016). Echolocation plays a vital role in detecting the water surface and guiding safe manoeuvres (Siemers *et al.*, 2010; Russo *et al.*, 2012; 2016). When drinking, bats must decelerate to contact the water surface, and they typically fly very close to the surface: under such circumstances they may perceive a higher risk of potential predation, especially if drinking at artificially illuminated sites. Moreover, under lit conditions bats rely on vision more than in the dark, and this reduces their ability to avoid obstacles (Orbach and Fenton, 2010): hence ALAN might impair drinking manoeuvres. Artificial illumination of freshwater habitats, including rivers, streams, ponds and pools – all potentially important bat drinking sites – is becoming more common for safety or recreational reasons (e.g. along cycling and running paths), especially when urban areas and their lights flank or surround them (Moore *et al.*, 2006; Perkin *et al.*, 2011).

If ALAN affects drinking behaviour it may constitute a further significant, and so far completely overlooked stressor affecting bat survival and fitness. In our study we tested the effect of short-term artificial lighting at cattle troughs used by bats as drinking sites. We hypothesized that lit conditions will affect drinking by bats. Specifically, we predicted that 1) the number of bat species and drinking events will be lower under artificial illumination and 2) forest species – e.g. *Plecotus auritus*, *Barbastella barbastellus* and *Myotis* spp. – that rely on insect food available all night (thus not used to being active early to pursue crepuscular prey), inhabit dark habitats and fly relatively slowly, will be more affected by illumination than edge species (e.g. *Pipistrellus* spp.), which

largely feed by aerial hawking on crepuscular insects and are therefore better adapted to higher light levels (Mathews *et al.*, 2015). Slow flying species such as forest species are more vulnerable to light-dependent predation risk than fast-flying bats, and are more averse to ALAN, than edge or open space species (Stone *et al.*, 2015b). Because the same study sites were also moderately used for foraging, at least in the first hours of the night, we used the same experimental approach to test the effect of illumination on foraging activity.

Materials and methods

Fieldwork was carried out in July and August 2015 at the Abruzzo, Lazio and Molise National Park, in the Italian central Apennines. We conducted lighting experiments at four cattle troughs > 2 km apart where on summer evenings bats congregate by the hundreds to drink (Russo *et al.*, 2012; 2016). All sites were surrounded by a similar habitat dominated by mature beech forest and pastures and were located at 1220–1563 m a.s.l. Drinking sites were made of two structurally similar, adjoining watering troughs, ca. 6×1.5 m each. Each site was visited twice following a random order but experiments at the same site were replicated only after ≥ five days to minimise habituation effects. We illuminated drinking sites with a portable LED (high-brightness light-emitting diodes) outdoor light emitting normal white light (Fig. 1) obtained from McMantom (Italy). The lamp type we adopted is commonly used for outdoor artificial illumination. Its 48 high-power LEDs generated a light intensity of 6480 lm (4000-4500 K) at 25°C with a power rating of 32±2 W. The light was powered by a 12V (35A) car battery switched on manually. One operator switched it on and off when needed and then moved away from the drinking site. The light spectrum showed a bimodal spectral pattern typical of this kind of lighting system (Rowse *et al.*, 2016) with two peaks of relative luminous flux at 450nm and 590 nm (data provided by the producer). The system emitted neither audible nor ultrasonic noise (checked with a bat detector) so we were confident that only light could affect bat behaviour during the experiments. We chose LED lighting for our experiments

because the use of this technology for outdoor illumination is spreading due to its energetic benefit, and negative effects on bat activity have been recorded (Stone *et al.*, 2012). The light unit was placed on a pole at 3m above the ground either at half the length, or at one end of the pool depending on the presence of trees, and was oriented to illuminate the whole trough (Fig. 1). The pole was placed ca. 1 m from the trough's walls. In all cases we checked visually that the entire pool was well illuminated and that the lighting set did not interfere with the bats' ability to fly along the troughs.

Experiments began and ended 50.5 ± 8.1 min and 220.6 ± 7.6 min after sunset respectively. We adopted a paired design in which comparisons were made between two 10-min sample intervals separated by a 5-min (dark) pause. Light was switched on during one sample interval, off in the other (representing the control). The sequence of lit and dark treatments followed a random order. Each night we recorded bat drinking activity over 5 sample interval pairs separated by a 10-min (dark) pause. We assumed this pause to be long enough to regard pairs as independent of each other. At the beginning of each sample interval we measured ambient temperature with a digital thermometer to the nearest 0.1 C, illuminance (in lux) at ground level with a Delta Ohm photo-radiometer (spectral range 450–760 nm, operational range 0–200,000 lux, resolution ≤ 200 lux = 0.1; > 200 lux = 1) and estimated wind speed according to the Beaufort scale. On each evening experiments were started when light intensity was ≤ 0.1 lux. When the light unit was on, mean light intensity ± SD was 48.6±1.4 lux.

We recorded bat echolocation calls with a Pettersson D1000X bat detector, which continuously sampled in the real-time mode (sampling rate 384000 Hz). Recordings were saved onto 4 Gb flashcards. The bat detector microphone was placed on the edge of each water trough at ca. half of its length and directed toward its centre. The main detector's unit was connected to the microphone by a 5-m long cable so that the operator sat quietly at a distance from the flight path used by approaching bats and did not interfere with them (Fig. 1). Drinking bats broadcast a rapid sequence

of echolocation calls – most probably needed to ensure a safe drinking manoeuvre – termed "drinking buzz" (Russo *et al.*, 2012; 2016; Griffiths, 2013). Each drinking buzz we recorded was counted as a drinking event. In most cases drinking buzzes were associated with a clear splashing sound produced by the bat breaking water surface. Because cattle troughs attract insects and may sustain moderate feeding activity (D. Russo, pers. obs.), we also counted feeding buzzes to estimate the potential effects of artificial illumination on foraging attempt rates. Feeding buzzes differ structurally from drinking buzzes so distinction between such call sequences was easy (Russo *et al.*, 2016). Although feeding buzzes only represent prey capture attempts, their numbers at feeding sites are representative of prey availability and may be used to estimate foraging rates (e.g. Russo and Jones, 2003). Hereafter we speak of "drinking" or "foraging" activity referring respectively to the numbers of drinking or feeding buzzes recorded during the experiments.

We used sound analysis to identify bats to species. BatSound 4 (Pettersson Elektronik AB, Uppsala) was employed to generate spectrograms with a 512-pt FFT Hamming window, 85% overlap (providing a 1120 Hz frequency resolution). For species identification, we analysed one good quality echolocation call selected from the sequence of pulses preceding the buzz. Species identification was carried out employing the quadratic discriminant function analysis (DFA) with cross validation carried out by Russo and Jones (2002) for Italian bat species and modified as in Russo *et al.* (2012), which was conducted in the same area. These functions only comprised species constituting >1% of bats caught at the experimental sites in summers 2000–2015. Probability of correct classification ranged between 0.69-1.00: the response was accepted for species whose probability of correct classification > 0.80. In the remaining cases (probably almost all *M. mystacinus*, with few or no *M. brandtii*, *M. alcathoe* and *M. bechsteinii*) calls were classified to the genus level. Two operators (NL, LA) also carried out a visual check of all recordings in BatSound and in all cases their diagnosis matched that provided by the DFA.

Preliminary data exploration carried out by correlation showed no significant effects of temperature or wind speed on drinking or foraging activity, so these factors were discarded from further analyses. We checked that data residuals conformed to normal distribution with a Ryan-Joiner test. To assess differences in the numbers of drinking and feeding buzzes we developed repeated measures general linear models (RMGLMs) using treatment (light or dark) and sample interval as fixed effects and site, date and paired sampling interval as random effects. For the analysis, intervals were numbered progressively from 1-5 so for each of them the number reflected the time at which recordings were made. Only species for which > 25 drinking or feeding buzzes were recorded were used for analysis. RMGLM results shown in text comprise F and P values as well as β coefficients, whose signs indicate a positive (+) or negative (-) effect of a given factor on bat activity. Models were developed in R 2.13.2 and significance was set at $P<0.05$.

Results

Overall, we recorded 2549 drinking events from 12 species or species groups (Table 1). The commonest species at drinking sites was *B. barbastellus*, the rarest was *Rhinolophus hipposideros*. *Myotis* calls were identified to species respectively in 21.5% of drinking events and 7.2% of foraging attempts, otherwise they were only classified to the genus level (Table 1). All species except *P. auritus*, *Pipistrellus pygmaeus* and *R. hipposideros* drank when drinking sites were illuminated. Feeding buzzes, albeit much rarer than drinking buzzes (n = 385), were recorded from 10 species or species groups. Due to sample size, quantitative analysis of feeding buzz numbers was only possible for *B. barbastellus*, *Pipistrellus pipistrellus*, *Pipistrellus kuhlii* and *Myotis* bats. Feeding buzzes were not recorded for *P. auritus* and *R. hipposideros* (Table 1).

We restricted quantitative analyses of drinking events to seven species and the whole *Myotis* dataset for which sample size was sufficiently large (Fig. 2). When all species were lumped together, treatment had an effect on drinking ($F_{1, 76} = 40.3$, $P < 0.0001$), but not foraging ($F_{1, 76} = 0.5$, n.s.)

activity (Figs 2, 3). The number of drinking buzzes recorded in lit intervals was significantly lower than in the dark (β = - 30.24). Interval (expressing the time recordings were made over each session) had a significant effect on both drinking ($F_{1, 76}$ = 20.4, P < 0.0001, β = -7.6) and foraging ($F_{1, 76}$ = 4.4, P < 0.05, β = -0.81) activities, which decreased over time. Species richness (expressed as the number of species recorded drinking and / or foraging) also decreased when light was switched on ($F_{1, 76}$ = 28.4, P < 0.0001, β = -2.05), as well as over time ($F_{1, 76}$ = 10.0, P < 0.005, β = -0.43).

Effects on forest species

B. barbastellus decreased drinking activity during lit intervals ($F_{1, 76}$ = 9.5, P < 0.01, β = -9.13) but not over time ($F_{1, 76}$ = 2.7, n.s.) (Fig. 2). On the contrary, the species' foraging activity (Fig. 3) increased in response to lighting ($F_{1, 76}$ = 9.7, P < 0.005, β = 1.02) and did not change over time ($F_{1, 76}$ = 1.5, n.s.). An even stronger negative effect of light on drinking activity was recorded for *P. auritus* (light vs. dark, $F_{1, 76}$ =17.7, P < 0.0001, β = -1.93) while time had no detectable effect (interval, $F_{1, 76}$ = 0.1, n.s.) (Fig. 2). Among *Myotis* species, *M. nattereri* drinking buzzes in lit intervals were rarer than in the dark ($F_{1, 76}$ = 5.9, P < 0.05, β = -1.95) and also their number decreased over time ($F_{1, 76}$ =8.6, P < 0.005, β = -0.83) (Fig. 2). For *Myotis emarginatus* (Fig. 2), only two drinking events in lit intervals were recorded vs. 69 in dark intervals (Table 1). However, between-site variation was large (i.e. drinking events were unevenly distributed across sites), as much as drinking activity did not change significantly according to treatment ($F_{1, 76}$ = 4.6, n.s.) but only decreased over time ($F_{1, 76}$ = 4.6, P < 0.05, β = -0.68).

Drinking ($F_{1, 76}$ = 32.6, P < 0.0001, β = -11.95) but not foraging ($F_{1, 76}$ = 0.8, n.s.) activity of *Myotis* bats decreased in lit intervals (Figs 2, 3), while both declined over time (drinking, $F_{1, 76}$ =28.1, P < 0.0001, β = -3.93; foraging, $F_{1, 76}$ = 28.5, P<0.0001, β = - 0.88).

Effects on edge species

P. pipistrellus showed no response to treatment or interval over drinking (light vs. dark, $F_{1, 76}$ =2.4, n.s.; interval, $F_{1, 76}$ = 21.7, n.s.) or foraging (light vs. dark, $F_{1, 76}$ =0.0, n.s.; interval, $F_{1, 76}$ = 0.1, n.s.) activity (Figs 2, 3). *Pipistrellus kuhlii* showed a different pattern (Figs 2, 3): while drinking activity slightly but significantly decreased in response to artificial light ($F_{1, 76}$ =5.8, P<0.05, β = -0.04) the number of feeding buzzes increased ($F_{1, 76}$ =7.4, P<0.05, β = 1.34). In neither case did interval have an effect, i.e. their activity remained stable over time (drinking, $F_{1, 76}$ =0.1, n.s.; foraging, $F_{1, 76}$ =1.0, n.s). Finally, the number of *Hypsugo savii* drinking buzzes (Fig. 2) declined in response to light ($F_{1, 76}$ = 5.0, P < 0.05, β = -1.00) but not over time ($F_{1, 76}$ = 0.02, n.s).

Discussion

The effects of lighting at drinking sites

We demonstrated for the first time that drinking bats are negatively affected by ALAN. Specifically, our study established that bat behaviour is significantly affected by a short exposure to artificial illumination, and that ALAN affects drinking behaviour much more than foraging behaviour. Although forest species were especially affected, our prediction that adverse reactions to ALAN would be restricted to them was only partly confirmed because some supposedly "tolerant" species also avoided drinking at lit sites. Five out of seven bat species, and the *Myotis* group reduced drinking activity when the drinking site was illuminated. Typical forest species such as *B. barbastellus* and *P. auritus* showed a strong adverse reaction to lighting when drinking. These bats are slow-flying moth hunters (Rydell *et al.*, 1996; Sierro and Arlettaz, 1997) that adopt respectively a "stealth" echolocation strategy (Goerlitz *et al.*, 2010) or passive listening (Anderson and Racey, 1993) to capture tympanate moths, insects capable of avoiding most other bat species by hearing

and responding to echolocation calls. Noticeably, *B. barbastellus* increased foraging activity when sites were illuminated. This is in agreement with previous work showing that when foraging or commuting this bat is not particularly sensitive to artificial light (Lacoeuilhe *et al.*, 2014) and may occasionally hunt near street lamps (Rydell, 2006; Ancillotto *et al.*, 2014). We speculate that *B. barbastellus* is more wary when drinking than when commuting or foraging, which would explain its avoidance of illuminated drinking sites. Although moderate light might in theory favour foraging in *P. auritus* because of its partial reliance on vision to detect and capture prey (Eklöf and Jones, 2003), in our experiments this species was absent from all recordings made when light was on. Adverse reactions to illumination of commuting or foraging bats were recorded by Lacoeuilhe *et al.* (2014) who classified *P. auritus* as intolerant to ALAN. A study assessing the different impact of full-time vs. part-time lighting on bats (Azam *et al.*, 2015) showed a non-significant reduction in the number of *Plecotus* spp. passes in the former condition relative to dark, while activity at part-night lighting sites was greater than at the unlit or full-night lighting sites, probably in response to increased prey availability (Azam *et al.* 2015).

Light also decreased drinking activity in *M. nattereri*, another gleaner (Swift and Racey, 2002) that frequently forages in forests (Smith and Racey, 2008). Based on previous long-term capture work at our study sites we are confident that unidentified *Myotis* passes mostly corresponded to *Myotis mystacinus* with few or no *M. brandtii*, *M. alcathoe* and *M. bechsteinii* (Russo *et al.*, 2012). These *Myotis* species are strongly linked to forests, often feeding on moths or gleaning prey from substrates (Taake, 1992; Siemers and Swift, 2006; Lučan *et al.*, 2009; Danko *et al.*, 2010; Buckley *et al.*, 2013), so their adverse response to light fits with the pattern described for the other moth-eating and/or gleaning species (*Plecotus* and *Barbastella*). Foraging *Myotis* bats were not affected by lighting, reinforcing the hypothesis that bats are especially sensitive when drinking. Although data suggested a negative effect of ALAN on drinking *M. emarginatus*, the trend was not supported statistically. This species often hunts in forest where it gleans large numbers of spiders (Flaquer *et*

al., 2008; Goiti *et al.*, 2011), so it is potentially sensitive to ALAN. Our results should be treated with caution as bright artificial illumination at roost sites has detrimental effects on this species (Boldogh *et al.*, 2007).

P. pipistrellus activity conformed to our prediction that when drinking edge specialists are more tolerant to ALAN. Although the species commonly hunts around street lamps (Arlettaz *et al.*, 2000), foraging activity showed no response to light. In *P. pipistrellus*, however, the response to artificial illumination may be site-specific (Mathews *et al.*, 2015) or depend on the type of illumination used (Blake *et al.*, 1994). For instance, Mathews *et al.* (2015) concluded that on a landscape scale ALAN has negative effects on the distribution of *P. pipistrellus* albeit local increases are possible at sites characterized by good tree cover. Noticeably, the same prediction was not confirmed by *P. kuhlii*, whose drinking activity declined under artificial illumination whereas foraging activity increased (see also Polak et al., 2011) as we observed in *B. barbastellus*. The result is especially interesting because *P. kuhlii* is an urban exploiter (Ancillotto *et al.*, 2015; Russo and Ancillotto, 2015; *Ancillotto et al.*, 2016) that forages very frequently near street lamps, especially those emitting white light (Barak and Yom-Tov, 1989; Tomassini *et al.*, 2014). The selective increase in skull size recorded in Italy after the Second World War has been related to the boost of the country's artificial illumination occurring since then, perhaps representing a microevolutionary response to the need of handling larger prey (moths) caught only at artificially illuminated sites (Tomassini *et al.*, 2014). Moreover, the productivity of *P. kuhlii* maternity colonies in suburban areas of Italy increased under increasing levels of artificial illumination surrounding roost sites (Ancillotto *et al.*, 2015). Negative reactions to illumination were also exhibited by drinking *Hypsugo savii*, another bat common in urban areas (Russo and Jones, 2003; Řehák, 2010; Uhrin et al., 2016). For *H. savii* too we failed to record enough feeding buzzes to explore the response of foraging bats.

Temporal effects on bat richness and activity did not show the same patterns in all cases and responses to light were species-specific. Factors such as distance travelled from roosts to drinking

sites, or changes in food availability over time may have influenced these findings. Many bats are likely to have reached our study sites to drink soon after leaving the roost and then probably moved elsewhere to forage, which would explain why both species richness and total drinking activity decreased over time. The decrease in drinking activity was mostly restricted to *Myotis* bats, however, whereas other species showed no trends. Bats whose roosts are far from drinking sites may reach the latter later at night: for instance, several *B. barbastellus* radiotracked in one of the study sites in previous work (Russo *et al.*, 2004) travelled for over 2 km from their roosts to reach cattle troughs. At least for species foraging at cattle troughs, decreased insect activity at lower temperatures (Williams, 1961) or progressive food depletion might have caused the temporal decline in the numbers of feeding buzzes.

Further work will be needed to test the effects of prolonged lighting, which might promote habituation (Thompson and Spencer, 1966). Moreover, we are aware that we carried out our experiment at a limited number of sites, and that results obtained at small artificial drinking sites such as those we used for our experiments may not match fully the conditions found at larger water bodies such as lakes. When large lakes are artificially illuminated, lighting often affects only part of water surface, leaving some areas unlit and still suitable for drinking. However, working at sites of limited size allowed us to manipulate light conditions over the entire water surface and obtain detailed sound recordings otherwise difficult to achieve – drinking buzzes are generally weak and directional (Russo *et al.* 2015) so they may go overlooked at large recording sites. Moreover, since we worked on free subjects in a natural set we are confident that our experimental conditions were more representative than those of tests done on captive subjects. Since we limited our experiment to LED technology and normal white light whose intensity was similar to that defined as "high" by Stone *et al.* (2012), we encourage exploring the effects of other lighting technologies (Stone *et al.*, 2015b), light colours (Mathews *et al.*, 2015; Spoelstra *et al.* 2015), and further light intensities to test whether other types of illumination have milder or no influence on bat drinking behaviour.

The fact that drinking bats were even less tolerant of lighting than foraging bats, including species that commonly exploit artificial illumination for foraging, makes the monitoring of this behaviour a priority to evaluate the actual effects of lighting at water sites. Forest bats are especially at risk: in our experiments certain species, such as *P. auritus*, were excluded from lit drinking sites. Besides affecting aquatic organisms (Perkin *et al.*, 2011; Becker *et al.*, 2013) the ever growing illumination of riversides and lake shores as well as of artificial structures such as dams, jetties and wharfs may have already played a major negative role for bat conservation.

Our study adds a new dimension to the issue of ALAN on bats, a well-known threat for bats in foraging and commuting areas (e.g. Stone *et al.*, 2009; 2015b; Mathews *et al.*, 2015; Rowse *et al.*, 2016). This study highlights a previously overlooked negative effect of ALAN on bats that may have important conservation repercussions given the overwhelming value drinking water has for these mammals (Korine *et al.*, 2016). We remark that responses may differ also among bat species from the same genus or ecological guild (Davidson-Watts *et al.* 2006, Ashrafi *et al.* 2013). Given the importance of watersites for bat feeding (e.g. Russo and Jones, 2003; Salvarina, 2016) and drinking (Korine *et al.*, 2016), artificial illumination at such habitats should be strictly forbidden or, if unavoidable (e.g. for safety reasons), appropriate mitigation or compensation measures (such as creation of alternative drinking sites) should be adopted to minimize effects on bats. The duration of illumination might be reduced through part-night lighting (PNL), as suggested to help limit the impact of ALAN on nocturnal wildlife (Gaston et al. 2012). Specifically, at drinking sites illumination might be restricted to the last part of the night since most bat drinking activity occurs over the first hours after sunset. In our study, bat drinking activity quickly increased once dark was re-established, so intelligent lighting schemes in which light is switched on automatically only when needed (Stone et al. 2015a) might mitigate the impact of ALAN on drinking behaviour. Other mitigation options might be offered by the reduction of trespass of lighting or the creation of light

exclusion areas to establish dark refuges (Stone et al. 2015a), providing undisturbed corridors and drinking spots for bats.

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Table 1. Total numbers of bat drinking and feeding buzzes listed by species or species group recorded under two experimental treatments (artificial lighting vs. dark) at four cattle troughs in central Italy. Unidentified *Myotis* include all *Myotis* passes other than those classified as *M. emarginatus* and *M. nattereri.*

Species	Drinking buzzes			Feeding buzzes		
	Light	Dark	Total	Light	Dark	Total
Barbastella barbastellus	306	584	890	16	9	25
Unidentified *Myotis*	134	599	733	68	59	127
Pipistrellus pipistrellus	165	240	405	42	40	82
Pipistrellus kuhlii	64	116	180	62	36	98
Myotis nattereri	25	105	130	2	3	5
Plecotus auritus	0	78	78	0	0	0
Myotis emarginatus	2	69	71	0	5	5
Hypsugo savii	10	39	49	2	5	7
Nyctalus leisleri	5	4	9	2	6	8
Pipistrellus pygmaeus	0	2	2	3	1	4
Rhinolophus hipposideros	0	1	1	0	0	0
Unidentified	1	0	1	19	5	24
Overall N events	712	1837	2549	216	169	385

Figure captions

Figure 1. Experimental light at a cattle trough used for drinking by bats in the central Italian Apennines

Figure 2. Block diagrams showing mean differences (± standard deviation) in drinking activity at four cattle troughs in Central Italy during paired dark (dark shaded blocks) vs. lit (light shaded bar) intervals. Intervals I1 and I5 respectively represent the first and last sample paired sample intervals for a recording evening. C= overall drinking activity; Bb = *Barbastella barbastellus*; Msp = all *Myotis* bats (unidentified *Myotis* + *M. emarginatus, M. nattereri*); Pa = *Plecotus auritus*; Mn = *Myotis nattereri*; Me = *Myotis emarginatus*; Hs = *Hypsugo savii*; Pk = *Pipistrellus kuhlii*; Pp = *Pipistrellus pipistrellus*. Bat and prey silhouettes represent the two different foraging styles typical of forest (gleaning) and edge (aerial hawking) bat species.

Figure 3. Block diagrams showing mean differences (± standard deviation) in foraging activity at four cattle troughs in Central Italy during paired dark (dark shaded blocks) vs. lit (light shaded bar) intervals. Intervals I1 and I5 respectively represent the first and last sample paired sample intervals for a recording evening. C= overall foraging activity; Bb = *Barbastella barbastellus*; Msp = all *Myotis* bats (unidentified *Myotis* + *M. emarginatus*, *M. nattereri*); Pk = *Pipistrellus kuhlii*; Pp = *Pipistrellus pipistrellus*. Bat and prey silhouettes represent the two different foraging styles typical of forest (gleaning) and edge (aerial hawking) bat species.